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Original Article

Patterns of BCR/ABL Gene Rearrangements by Fluorescence in Situ Hybridization (FISH) in Chronic Myeloid Leukaemia

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Abstract

Background: Chronic myeloid leukaemia (CML) is myeloproliferative disorder. The t (9;22), encoding the formation of the BCR/ABL1 fusion gene, is the hallmark chromosomal abnormality detected in CML. This translocation is detectable by RT-PCR or Conventional Karyotyping in 95% patients of CML. However, in 5-10% of cases, the patients fail to demonstrate the presence of Philadelphia chromosome at diagnosis, despite the presence of the BCR/ABL gene rearrangement.
Objective: To study the atypical BCR/ABL gene rearrangement patterns by Fluorescence in situ hybridization (FISH) in patients of CML and to determine their clinico-haematological characteristics and response to treatment.
Study design, settings and duration: A prospective study, conducted at the department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, from 1st June 2017 to 30th May 2018.
Subjects and Methods: The study group included all patients diagnosed as CML based on WHO criteria. RT-PCR and conventional cytogenetics were done on all samples. All patients negative on PCR and cytogenetics were then analyzed by FISH. Interphase FISH analysis on at least 500 nuclei, using a commercially available BCR/ABL1 dual colour dual fusion probe, was performed on initial presentation on bone marrow cells prepared according to standard cytogenetic techniques.
Results: Two hundred three diagnostic samples of CML were analyzed of the total, 128 (63%) patients were male and 75 (36.9%) were female. Median age of diagnosis was 38 years. On FISH analysis, atypical signals patterns were observed in 16 (7.9%) patients. These atypical patterns included atypical BCR/ABL gene rearrangements with co-existence of der (9q) and der (22q) deletion in 13 (6.4%) patients and mBCR/ABL gene rearrangement associated to 9q deletion of non-rearranged chromosome 9 in 3 (1.5%) patients.
Conclusion: Atypical patterns in CML though rare, however, should be kept in mind when morphological diagnosis supports CML. FISH, in conclusion, is a sensitive, specific and efficient tool to infer these BCR/ABL gene rearrangement patterns.
Key words: Chronic myeloid leukaemia, flourescent in situ hybridization (FISH), BCR/ABL gene rearrangement patterns.

Introduction

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder predominantly of adult life, having a male preponderance.[1] These patients have variable presentations.[2] Some are completely asymptomatic, their disease being diagnosed incidentally on routine blood CP while others may have symptoms due to anemia or splenomegaly.[3] CML is usually diagnosed on peripheral blood film but the detection of the BCR-ABL fusion transcript by molecular techniques is necessary for establishing a definitive diagnosis.[4] Bone marrow examination is, however, essential to provide sufficient material for cytogenetics and also for assessment of the stage of the disease.[3]

Three phases of the disease have been identified.[5] Most of the patients present in the chronic phase demonstrating a bimodal peak and less than 5% blasts. Accelerated phase is more advanced disease defined by WHO as increasing splenomegaly, increasing counts or blasts 5-19% or

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CAH conceptualized the project. KI performed data collection & literature search. RM did the statistical analysis. Drafting, revision and writing of manuscript were done by HSM.

presence of additional cytogenetic abnormalities. The blast phase is characterized by more than 20% blasts.[6]

The presence of the BCR-ABL1 fusion gene in CML is an essential feature not only for establishing diagnosis but also for monitoring response to treatment.[7] Classically, in majority of the patients with chronic myeloid leukaemia, this BCR-ABL1 fusion gene results from a reciprocal translocation involving the long arms of chromosome 9 and chromosome 22. The abnormal chromosome 22 is designated the Philadelphia chromosome.[8] This translocation is detected by RT-PCR or conventional karyotyping.[9] However, in 5-10% cases, the patients fail to demonstrate the presence of the Philadelphia chromosome at diagnosis, despite the presence of the BCR-ABL1 fusion gene. This is because the BCR-ABL fusion gene can also result from variant and complex translocations and from cryptic chromosomal rearrangements.[10] Proposed mechanisms of these atypical gene rearrangements , though not fully characterized, involve a cryptic insertion between or submicroscopic deletions of chromosomes 9 and 22 or a classic t (9:22) followed sequentially by a reverse translocation. Additionally, the involvement of a third chromosome can result in complex rearrangements which may not yield the BCR-ABL1 fusion gene.[11]

Conventional cytogenetics can identify the presence of the Philadelphia chromosome, however, is unable to pick the cryptic insertions or submicroscopic deletions. Interphase fluorescent in situ hybridization has revolutionized the diagnosis as it can detect these variants with greater sensitivity and specificity.[12]

There are no studies available regarding the patterns of BCR-ABL1 gene rearrangements in CML in the Pakistani population. The rationale of this study was to characterize these patterns in our population and evaluate response to tyrosine kinase inhibitors to determine prognosis.

Materials and Methods

All subjects were elaborately apprised about the study and written informed consent was obtained. Over a span of 1 year, from 1st June 2017 to 31st May 2018, this study was done with cross-sectional design in the Department of Haematology, AFIP, Rawalpindi. Newly diagnosed adult patients above the age of 18 years were enrolled in the study. Patients on treatment of any kind were excluded. Patients who did not show adequate yield on cytogenetics were also excluded from the study.

After detailed history and examination, CBC was performed on Sysmex XE-5000. Peripheral blood film was formed followed by bone marrow examination and patients were diagnosed as having Chronic Myeloid leukemia (CML) on the basis of morphology.

RT-PCR for BCR-ABL fusion gene was performed on ABI 7500 RT-PCR analyzer. Heparinized bone marrow samples were collected and conventional cytogenetic analysis was performed by Giemsa trypsin banding technique.We analyzed at least twenty metaphases by Cytovision semi-automated image analysis system.

FISH studies were performed on all samples negative for BCR-ABL by RT-PCR and negative for Philadelphia chromosome on routine cytogenetics. For Interphase FISH analysis, specimens were processed by standard methods. Metasystems BCR-ABL1 dual colour dual fusion probe was used on the slide. The analysis of about 500 nuclei was performed and interpretation was done using automated Metasystems analysis system.

Patients were started on Imatinib therapy 400mg OD as per standard protocol. General physical examination and blood counts with differentials were assessed at 3 and 6 months, respectively to see the haematological response. Complete haematological response was defined as non-palpable spleen, normalization of blood counts, absence of basophils in differential count and no immature cells on peripheral smear. While presence of one or more of these but not all was defined as partial haematological response.

The data was analyzed by SPSS 20. Quantitative variables i.e. age, haemoglobin, platelet count and absolute basophil counts have been presented by mean±SD. Frequency and percentage was used for the expression of qualitative variables.

The ethical approval was taken from Ethical review committee of AFIP, Rawalpindi.

Results

A total of 203 newly diagnosed patients of chronic myeloid leukaemia were enrolled. Among the patients, median age was 38 years. Of these, 128 (63%) were males while 75 (36.9%) were females. Among them, 187 (92.1%) patients were positive for BCR-ABL fusion gene by RT-PCR and Philadelphia chromosome on conventional cytogenetics (Figure-1). However, 16 (7.9%) patients were negative on both RT-PCR and cytogenetics but interphase FISH studies revealed atypical rearrangements of gene BCR/ABL. These

patterns included atypical BCR/ABL gene rearrangements with co-existence of der (22q) and der(9q) and deletion in 13 (6.4%) patients and this gene rearrangement associated with deletion of 9q of chromosome 9 in 3 (1.5%) patients as shown in Figure-2.

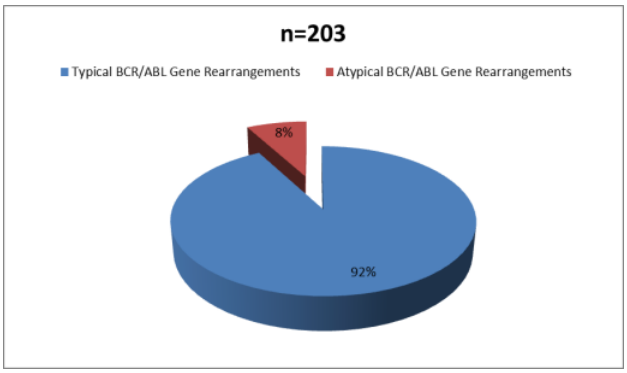

Figure 1: Patterns of BCR/ABL gene rearrangements.

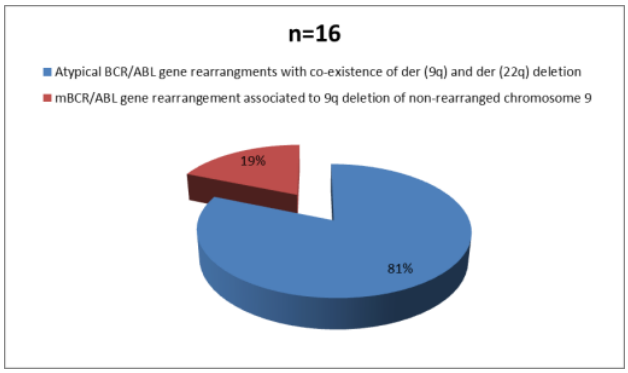

Figure 2: Distribution of atypical BCR/ABL gene rearrangements.

The patients having these atypical patterns had a median age of 44 years. Of these, males were 7 (43.8%) while 9 (56.3%) were females. We studied the clinico-haematological characteristics of these patients with atypical BCR/ABL gene rearrangement patterns. Five (32.5%) patients had constitutional symptoms at the time of presentation and 4 (25%) had massive splenomegaly. The median WBC count was $36x10^9$/l, haemoglobin level was 8.2 g/dl while the platelet count was $296x10^9$/l. At the time of presentation, initial workup included bone marrow examination. Eleven (68.75%) patients were in the chronic phase at the time of presentation while 4 (25%) patients presented in the accelerated phase and one (6.25%) patient was in blast phase. On trephine biopsy, three (18.75%) patients had fibrosis more than grade 2. We have compared the clinico-haematological characteristics of patients of chronic myeloid leukaemia with typical and atypical BCR/ABL gene rearrangements (Table).

Table: Comparison between typical and atypical bcr/abl gene rearrangements.

	Typical BCR/ABL Gene Rearrangements	*Atypical BCR/ABL Gene Rearrangements*
Median age (years)	38 years	44years
Gender male	68.4%	43.8%
Clinical		
Constitutional symptoms present	80.7%	31.3%
Massive splenomegaly	57.8%	25%
Blood counts		
Median WBC	$92x10^9$/l	$35x10^9$/l
Median Hb	9.5 g/dl	8.2 g/dl
Median platelet	$378x10^9$/l	$296x10^9$/l
Phase of CML		
Chronic phase	97.3%	68.8%
Accelerated phase	2.7%	25%
Blast phase	-	6.25%
Reticulin fibrosis		
Fibrosis $\geq$ grade 2	76.5%	18.8%

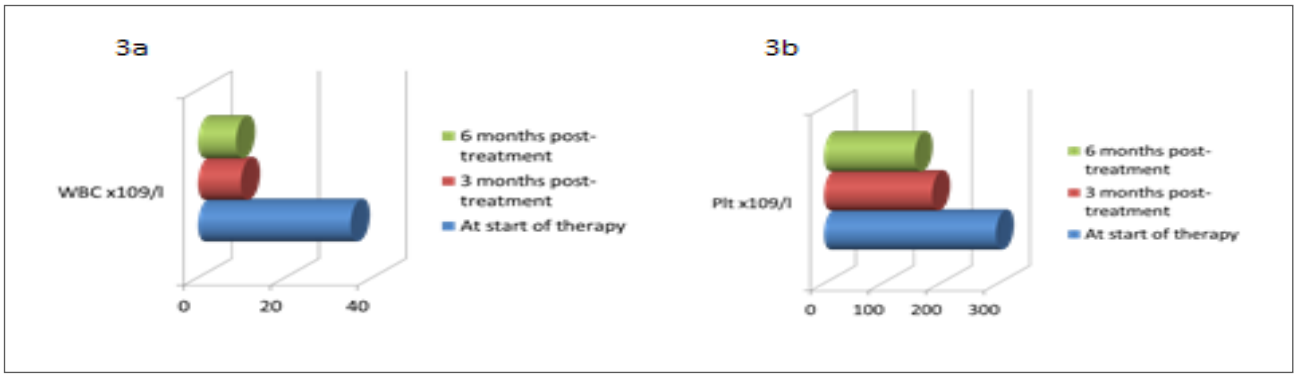

Figure 3a &3b: Description of white blood cells and platelets at start of therapy and post treatment.

Patients with atypical BCR/ABL gene rearrangements were started on Tablet Imatinib 400mg once daily. They were followed up by clinical examination and blood counts at 3 months and 6 months to assess response to treatment. One patient was lost to follow–up. Figure-3a & 3b show the WBC and platelet counts at 0, 3 and 6 months.

At end of six months, the spleen had regressed in all patients except 2. On peripheral smear examination, one patient had immature cells. According to the responses defined, 12 had achieved complete haematological response while 3 had shown a partial response. This is shown in Figure-4.

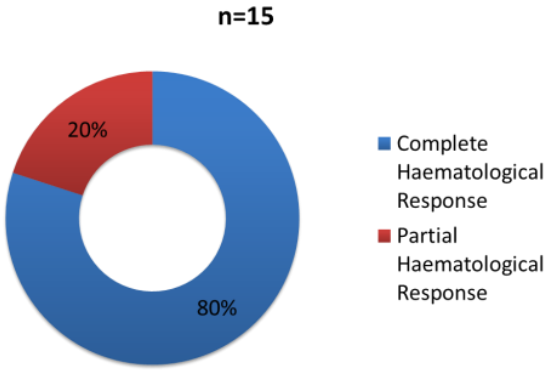

Figure 4: Haematological response.

Discussion

The t (9;22) encoding the formation of the BCR/ABL1 fusion gene is the hallmark chromosomal abnormality detected in chronic myeloid leukaemia. Its presence is thus essential to establish the diagnosis. In the past, conventional cytogenetics was the only diagnostic tool for the detection of t (9;22). However, over the years it was seen that in 5-10% of CML patients, conventional cytogenetics may fail to detect rearrangements of BCR/ABL gene. This led to the discovery that few CML cases carry 'masked' or 'atypical' or 'variant' translocations which can only be detected through molecular techniques such as FISH technique.

In developing countries like Pakistan, there are very few centers with facilities for cytogenetic and molecular studies. In AFIP, conventional cytogenetics and RT-PCR were performed for assessment of treatment response and determining minimal residual disease. This is very useful to identify these rare atypical BCR/ABL gene rearrangement patterns.

In our study, 16 (7.9%) patients had atypical BCR/ABL gene rearrangements on interphase FISH analysis. Marzocchiet al [13] has reported variant translocations in 5% of the CML patients enrolled in the GIMEMA Working Partyon CML trials in Italy. However, an Indian study conducted by Poonam P Jain [14] has reported a much higher frequency of atypical signal patterns. She reported a frequency of 26%. The atypical patterns observed in the Indian population include deletions of derivative 9 involving chromosome 9 sequences, chromosome 22 sequences, both; additional Philadelphia chromosome and more than one signal pattern while in our population only two variant patterns were observed. These were atypical BCR/ABL gene rearrangements with co-existence of der (22q) and der (9q) deletion and associated with deletion of 9q from non-rearranged chromosome 9.

The median age of our patients with variant translocations was 44 years while Marzocchiet al [13] has reported a much higher age of 52 yrs. He reported a male to female ratio of 1.7:1. This is in contrast to our findings as these atypical patterns in our study group were predominantly seen in females.

In a study conducted in Italy, 93% patients achieved complete haematological response while in our study 80% achieved complete haematological response.

In our population, atypical BCR/ABL gene rearrangement patterns were identified in 7.9% of patients with normal cytogenetics. The patterns observed were atypical BCR/ABL gene rearrangments with co-existence of der (9q) and der (22q) deletion and mBCR/ABL gene rearrangement associated to 9q deletion of non-rearranged chromosome 9. However much larger studies over a longer time frame are required to further establish our results.

Conflict of interest: None declared.

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